# REMARKS

## 1. Status of the Claims

Claims 1-8 stand pending. Claims 1-8 stand rejected. Claim 1 also stands objected to.

After entry of the present amendments, claims 1 and 8 stand amended, and claims 9-11 newly introduced. The amendments to the claims and the new claims are supported at least for example at ¶ [0009], at ¶¶ [0018]-[0024], and the originally filed claims. The claims have been amended without prejudice to, or disclaimer of, the canceled subject matter. Applicants reserve the right to file a continuation or divisional application on any subject matter canceled by way of amendment.

## 2. Acknowledgement of Priority

Applicants respectfully request that the Office acknowledge Applicants' claim for priority in the Office's next communication.

## 3. Information Disclosure Statement

Applicants submit an Information Disclosure Statement herewith. Applicants respectfully request consideration and acknowledgment of the listed references with the Office's next communication.

## 4. Objection to the Claim

Claim 1 stands objected to because the abbreviation "AN/TN" should be fully written out. Office Action at page 2. Applicants have amended claim 1 as indicated by the Examiner thereby mooting the objection. Support for the amendment can be found at least at ¶[0009]. Applicants respectfully request withdrawal of the objection and allowance of the claim.

## 5. Rejection of the Claims Under 35 U.S.C. § 112, Second Paragraph

### 5.1 Claims 1-8

Claims 1-8 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Office rejected claims 1-8 alleging that "[a] broad

range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim) is considered indefinite." Office Action at page 2.

Applicants have amended claim 1 such that only one range is listed thereby mooting the rejection. Support for the amendment can be found at least in the original claim. Additionally, the newly introduced claims 9-11, all of which depend upon the amended claim 1, should comply with the requirement of 35 U.S.C. § 112, second paragraph. Accordingly, Applicants request withdrawal of the rejection and allowance of the claims.

### 5.2 Claim 8

The Office rejects claim 9 as indefinite. Office Action at page 3. Applicants note that a call and message was left with the Examiner on December 30, 2008 to discuss claim 9. Claim 9 is newly introduced upon entry of the present amendments. Based on the discussion of "claim 9," Applicants assume absent the Examiner's confirmation, that the Office meant to discuss claim 8. Applicants have amended claim 8, as kindly suggested by the Office. Applicants thus believe that this rejection is accordingly mooted and can be withdrawn, and claim 8 allowed.

## 6. Rejection of the Claims Under 35 U.S.C. § 103(a)

### 6.1 Claims 1 and 3-8

Claims 1 and 3-8 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over **JP 3163127** [hereinafter "the '127 patent"] in view of **Yamanaka** (U.S. Pat. No. 3,882,635) [hereinafter "Yamanaka"]. The Office points out that there appears to be a common inventor between the Japanese patent application and the instant application. Office Action at page 4. From the perspective of 35 U.S.C. § 103(c), the fact that Y. Kakizono may be listed on both the '127 patent and the instant application is irrelevant and does not have to be addressed. For §103(c) to apply, the reference must be available under 35 U.S.C. § 102(e), (f), or (g), which in short requires that it be a U.S. application/patent; JP 3163127 is not a U.S. Application.

The Office alleges that the '127 patent "teaches a process for producing astaxanthin that comprises culturing green alga with an organic nitrogen source to obtain algal bodies in which the astaxanthin, which would be contained in lipids, has been stored…." Office Action at page 5. It is further asserted to allegedly teach that the "organic nitrogen used in the process is yeast

extract at least at a concentration of 2 g/L". *Id.* The reference also purportedly teaches that the culture can be performed under aerobic conditions and may be performed in a reaction under light or dark conditions. *Id.* The Office admits that the '127 patent does not teach a specific AN/TN ratio. Yamanaka is combined for allegedly teaching "a process for producing green algae wherein the alga is cultured with an organic nitrogen source, wherein the organic nitrogen source is peptone." *Id.* The Office assumes that the ordinary skilled artisan would have been motivated to alter the organic nitrogen source, because "Yamanaka teaches that green alga can be ground with a variety of nitrogen sources including peptone." Office Action at page 6. The Office further concludes that it would have been reasonable to achieve the claimed ratio via routine optimization.

Applicants traverse the rejection to the extent it applies to the amended claims. The Office concludes without reasoned analysis that (1) the ratio is obvious via routine optimization, and (2) optimization is necessary for organic nitrogen alone compared to any other media component. There is nothing in either reference, let alone their combination, that would suggest that the amount of organic nitrogen could be modulated to result in a good yield of astaxanthin by alga.

Applicants hereby point out that all the Abstract of the '127 patent teaches is a medium. The '127 patent does not relate to astaxanthin production whatsoever. There is no specific aspect of the medium that is indicated to be more important than the rest. The alga used is *Haematococcus pluvialis* NIES 144. The medium contained yeast extract (2.0 g/L); Na-acetate (1.2 g/L); L-asparagine (0.4 g/L); $MgCl_2 \cdot 7H_2O$ (984 μM); $FeSO_4 \cdot 7H_2O$ (36 μM); and $CaCl_2 \cdot 2H_2O$ (136 μM, pH 6.8). None of the conditions or reagents is indicated to be any more or less important than any other. Applicants further provide herein a partial translation of the '127 patent. *See* Annex 1. The '127 patent explicitly describes:

> ¶ [0010] Although the yeast extract is required, if an amino acid such as glycine and glutamine is used, growth of *Haematococcus pluvialis* equivalent to that in the case of using a yeast extract is observed even when no yeast extract is used.
>
> ¶[0022] Use of a basal medium specified in Table 1 without the yeast extract resulted in a low level of *Haematococcus pluvialis* growth; however, ***addition*** of an amino acid such as glycine and glutamine enabled recovery of a level of

> *Haematococcus pluvialis* growth, indicating that the amino acid could be substituted for the yeast extract.

(emphasis added). The '127 patent thus in fact ***teaches away*** from the present application. A skilled artisan aware of the disclosure of the '127 patent would have increased the AN/TN ratio to achieve maximal *Haematococcus pluvialis* growth, because a low AN/TN ratio (1) would have been expected to have had an extremely high tendency to foam, and (2) would have been difficult to achieve uniform mixing.

Yamanaka teaches in the abstract that the culture media is comprised of aqueous culture medium containing assimilable sources of carbon and nitrogen, inorganic salts, and minor organic nutrients. *See* Abstract. Similarly, nothing in Yamanaka places more importance on nitrogen sources or ranges than on any other component of the culture media. Additionally, Yamanaka does not relate to astaxanthin production whatsoever. Accordingly, the Office improperly applies Yamanaka in the present rejection.

The Office has failed to provide a reasoned explanation of why out of the itemized components of the two medias of the two references, the nitrogen source would be the one to modify, ***and*** that its modification would lead to an expectation of success for producing astaxanthin from alga, ***and*** enhancing astaxanthin production over medias in which the nitrogen source had not been so modulated. There is nothing in the references that demonstrates that would have suggested the possibility of improvement using the claimed combination over any other variation to the constituents in the media. Thus, Applicants conclude the Office has applied an improper picking and choosing of reference teachings with the assistance of hindsight analysis. *SmithKline Diagnostics, Inc. v. Helena Laboratories Corp.*, 859 F.2d 878, 886-87, 8 U.S.P.Q.2d 1468, 1475 (Fed. Cir. 1988) ("...cannot pick and choose among the individual elements of assorted prior art references to recreate the claimed invention. *See, e.g., Azko N.V. v. United States Int'l Trade Comm'n* , 808 F.2d 1471, 1481, 1 U.S.P.Q.2d 1241, 1246 (Fed. Cir. 1986), *cert. denied*, 107 S. Ct. 2490 (1987)."). Applicants point out that culturing conditions for any cell are not a matter of routine optimization, as there are numerous publications, books, and patents on the topic of culture media.

Applicants further direct the Office to [0006] of the Specification:

> Organic nitrogen sources such as high-protein nitrogen sources including defatted soy bean powder, in general, have an extremely ***high tendency to foam*** and contain large amounts of water-insolubles, so ***uniform mixing is difficult*** to achieve in a large, outdoor, open reactor; CSL (corn steep liquor) which is the liquid byproduct of sugar purification does not have uniform quality, especially in terms of the quantity of the microorganism that enters, and this is ***likely to induce microbial contamination*** in the outdoor, open reactor in which it is difficult to sterilize the medium

(emphasis added). The above section describes the drawbacks of using organic nitrogen sources, which generally have a low AN/TN ratio. Accordingly, a skilled artisan unaware of the present application would not have considered cultivating *Haematococcus pluvialis* in a medium with a low AN/TA ratio. The success as disclosed in the present application thus amounts ***unexpected*** results.

Additionally, the newly introduced claims 9-11, all of which depend upon the amended claim 1, stand non-obvious over the alleged prior art. Particularly, claims 9-10 recite algae in the genus *Haematococcus*. Yamanaka discloses culture media suitable for an alga of the genus *Prototheca*, which is taxonomically distinct from the genus *Haematococcus*. *See* Annex 2. Algae from the genus *Prototheca* are saprotrophic, *i.e.*, feeding on dead and decaying organic matter, because they do not contain chlorophyll. Algae from the genus *Haematococcus*, however, are known to be autotrophic organisms, because they have chlorophyll that enables the cells to photosynthesizing organic substance from $CO_2$.

Accordingly, Applicants assert that no *prima facie* case of obviousness has been adduced and the rejection should be withdrawn.

**6.2 Claims 1-8**

Claims 1-8 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over the '127 patent in view of Yamanaka as applied to claims 1 and 3-8, and further in view of **Tanaka** (WO 02/077105) [hereinafter "Tanaka"]. The '127 patent and Yamanaka are asserted for the reasons discussed *supra.* Tanaka is asserted for teaching "a method of extracting a lipid containing astaxanthin from ruptured algae." Office Action at page 7. Thus, the Office concludes that claim 2 is obvious in view of the combination of references.

Applicants traverse to the extent it applies to the amended claims. Tanaka does not teach or suggest the AN/TN ratio recited in the present claims. The Office has failed to adduce claim 2 as obvious given its failings in analyzing why the skilled artisan would have want to optimize the level of nitrogen at all, let alone that it, from all the other ingredients used in the culture media to grow the algae, would be the one to be optimized. Tanaka fails to cure these defects and overcome the teaching away of why a decreased AN/TN ratio could have been expected to work of the '127 patent and the Yamanaka reference as discussed *supra*. Accordingly, the combination of the three references cannot be found to teach or suggest any of claims 1-8, including claim 2. Similarly, the newly introduced claims 9-11, all of which depend upon the amended claim 1, stand non-obvious over the alleged prior art.

Accordingly, Applicants respectfully request withdrawal of the rejection and allowance of the claims.

## CONCLUSION

Should the Examiner have any questions or comments regarding Applicants' amendments or response, please contact Applicants' undersigned representative at (202) 842-8821. Furthermore, please direct all correspondence to the below-listed address.

If there are any other fees due in connection with the filing of this Amendment and Reply, please charge the fees to our Deposit Account No. 50-0573. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,
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# ANNEX 1

**Japanese Patent No. 3163127**

Translation(Part)

[0010]

Cultivation of *Haematococcus pluvialis* in the dark under aerobic conditions is performed as follows. The green alga *Haematococcus pluvialis* is a photosynthetic organism that is grown in the Nature with the aid of carbon dioxide and light energy. Thus, to culture the green alga *Haematococcus pluvialis* in the dark under aerobic conditions, it is necessary to use a medium in which a carbon source that can be substituted for carbon dioxide is contained in a sufficient amount to effect heterotrophism. Carbon sources that can be used include the conventionally known ones such as acetic acid, pyruvic acid, ethanol, and TCA-related organic acids. Examples of TCA-related organic acids include citric acid, alpha-ketoglutaric acid, succinic acid, fumaric acid, and malic acid. A medium for use contains a yeast extract in combination with one or two or more sources selected from the group consisting of the above carbon sources and nitrogen sources such as amino acids, including asparagine, glycine, and glutamine. Although the yeast extract is required, if an amino acid such as glycine and glutamine is used, growth of *Haematococcus pluvialis* equivalent to that in the case of using a yeast extract is observed even when no yeast extract is used. Examples

of preferred media include a medium containing 2.0 g/l of a yeast extract, 1.2 g/l of sodium acetate, 0.4 g/l of L-asparagine, 985 μM of $MgCl_2 \cdot 7H_2O$, 6 μM of $FeSO_4 \cdot 7H_2O_3$, and 136 μM of $CaCl_2 \cdot 2H_2O$, with pH 6.8. Cultivation is performed in the dark and under aerobic conditions at a temperature of 15°C to 25°C, preferably about 20°C.

[0019] Example 1

In a 200-ml flask, 100 ml of a basal medium specified in Table 1 was placed and then sterilized at 121°C for 15 minutes. A basal medium for maintenance was inoculated with *Haematococcus pluvialis* (*Haematococcus pluvialis* NIES 144) seeds that had been cultured separately, and then pre-cultured at 20°C for 4 days in the dark and in the light, for example at an illuminance of 1500 lux, under a 12-h light/12-h dark illumination cycle.

[0020]

Each of 100 ml media having the above-described composition was inoculated with 10 ml of the culture solution, and main culture was performed at 20°C for 8 days in the dark and in the light.

[0021]

Figure 1 shows changes in amount of carotenoid grown and produced in vegetative cells of *Haematococcus pluvialis* during the main culture in the dark or in the light. The amount of carotenoid was measured at an

absorbance of 480 nm. Thereafter, the carotenoid was extracted, and about 90% of the carotenoid was astaxanthin.

[0022]

*Haematococcus pluvialis* grew in the dark with the aid of acetic acid as a carbon source. Use of a basal medium specified in Table 1 without the yeast extract resulted in a low level of *Haematococcus pluvialis* growth; however, addition of an amino acid such as glycine and glutamine enabled recovery of a level of *Haematococcus pluvialis* growth, indicating that the amino acid could be substituted for the yeast extract. A suitable range of a carbon source concentration is up to 45 mM. *Haematococcus pluvialis* obtained in the dark contained chlorophyll and carotenoid pigments, and amounts of the pigments per cell were equivalent to those in *Haematococcus pluvialis* obtained in the light. The pigments were grown and accumulated in cells. A chlorophyll content was 10-20 mg/g by dry weight, and a carotenoid content was 10 mg/g by dry weight.

[0023]

[Table 1]

| | |
|---|---|
| Yeast extract | 2.0 g/l |
| Sodium acetate | 1.2 g/l |
| L-asparagine | 0.4 g/l |
| $MgCl_2 \cdot 7H_2O$ | 984 μM |
| $FeSo_4 \cdot 7H_2O$ | 36 μM |

| | |
|---|---|
| $CaCl_2 \cdot 2H_2O$ | 136μM |
| pH | 6.8 |

# ANNEX 2

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**Lineage** (full): root; cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; Chlorophyceae; Chlamydomonadales; Haematococcaceae

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o **Haematococcus** *Click on organism name to get more information.*

- **Haematococcus droebakensis**
- **Haematococcus lacustris**
- **Haematococcus pluvialis**
- **Haematococcus zimbabwiensis**

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**Lineage** (full): root; cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; Trebouxiophyceae; Chlorellales; Chlorellaceae

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- **Prototheca** *Click on organism name to get more information.*
  - **Prototheca blaschkeae**
  - **Prototheca moriformis**
  - **Prototheca stagnora**
  - **Prototheca ulmea**
  - **Prototheca wickerhamii**
  - **Prototheca zopfii**
    - **Prototheca zopfii var. hydrocarbonea**
    - **Prototheca zopfii var. portoricensis**
    - **Prototheca zopfii var. zopfii**

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